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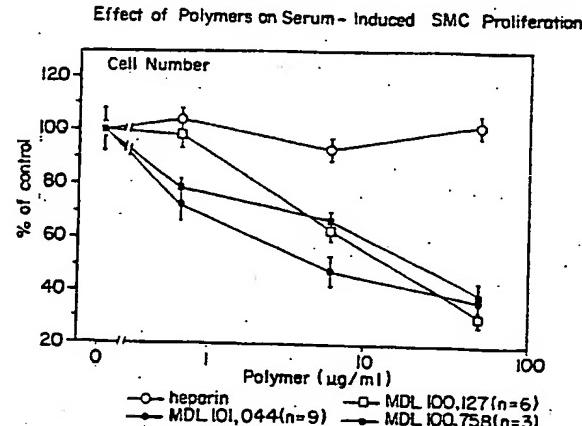
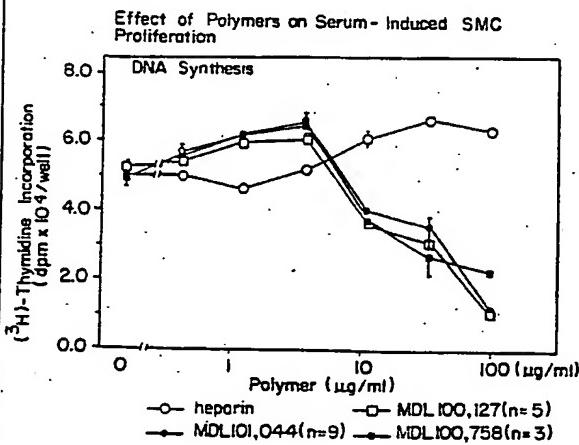
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(21) International Application Number: PCT/US93/06693		(74) Agent: NESBITT, Stephen, L.; Marion Merrell Dow Inc., 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH 45215-6300 (US).
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(71) Applicant: MERRELL DOW PHARMACEUTICALS INC. [US/US]; 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH 45215-6300 (US).		Published <i>With international search report.</i>
(72) Inventors: CARDIN, Alan, D.; 9903 Hunters Run Court, Cincinnati, OH 45242 (US). BUSCH, Steven, J.; 8820 Brooks Knoll Drive, West Chester, OH 45069 (US). MANO, Masayuki; 875-21, Tenjin, Izumo, Shimane 693 (JP).		

(54) Title: ANTIPIROLIFERATIVE OLIGOMERS



(57) Abstract

The oligomers of the present invention are polyureas or polyamides having a number average molecular weight of < 10,000. These oligomers are water-soluble and are pharmaceutically-acceptable. The oligomers are use:

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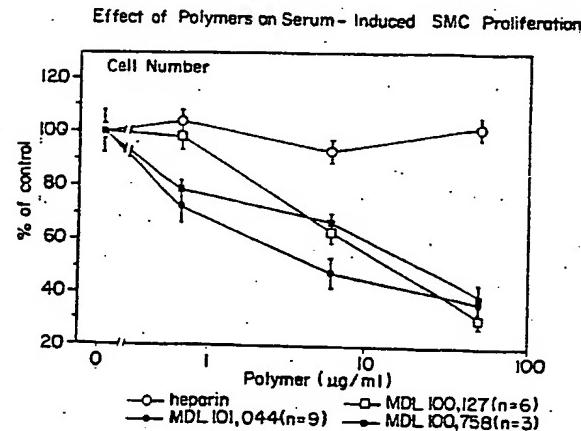
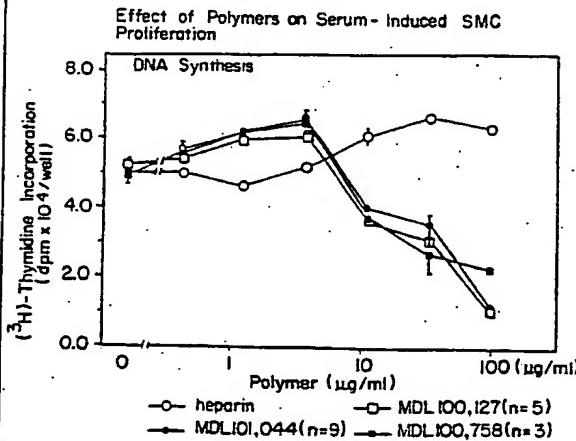
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(54) Title: ANTIPIROLIFERATIVE OLIGOMERS



(57) Abstract

The oligomers of the present invention are polyureas or polyamides having a number average molecular weight of < 10,000. These oligomers are water-soluble, have a rigid backbone with a predictable anion spacing, and are pharmaceutically-acceptable. The oligomers are useful for inhibiting the proliferation of smooth muscle cell.

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-1-

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ANTIPROLIFERATIVE OLIGOMERS

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The invention relates to certain anionic oligomers which are useful antiproliferative agents by virtue of their ability to inhibit smooth muscle cell proliferation. These compounds can be used in the treatment of, for 15 example, atherosclerosis.

Proliferation of smooth muscle cells in blood vessel walls occurs in response to vascular injury and in association with certain disease states. The proliferation 20 of these cells can lead to the formation of pathologic lesions, for example, atherosclerosis and postsurgical vascular restenosis. Various glycosaminoglycans including heparin have been reported to inhibit smooth muscle cell proliferation. However, long term therapy with heparin is 25 limited by its untoward side effects.

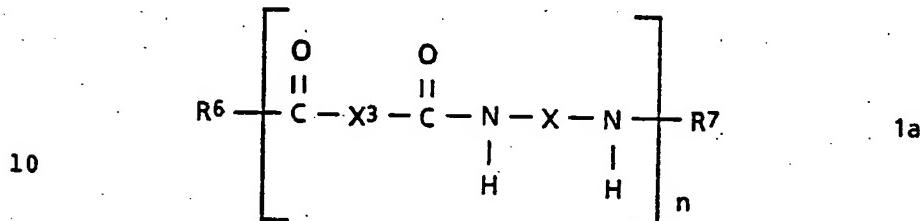
Applicants have discovered that a class of synthetic oligomers are heparinmimetic in that this class of oligomers inhibits smooth muscle cell proliferation in 30 culture and *in vivo*. The synthetic oligomers do not possess the liabilities of prolonged heparin therapy. Such oligomers would thus be useful in the treatment of a variety of diseases and conditions associated with the smooth muscle cell and with other cells exhibiting 35 unregulated cell proliferation.

-2-

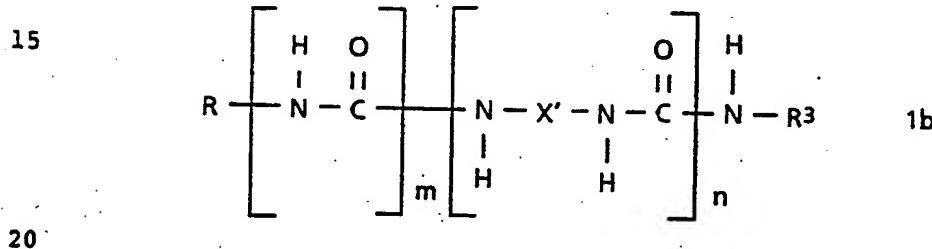
## SUMMARY OF THE INVENTION

This invention relates to the use of anionic polyamide and polyurea oligomers of formulae 1a and 1b, respectively,

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wherein

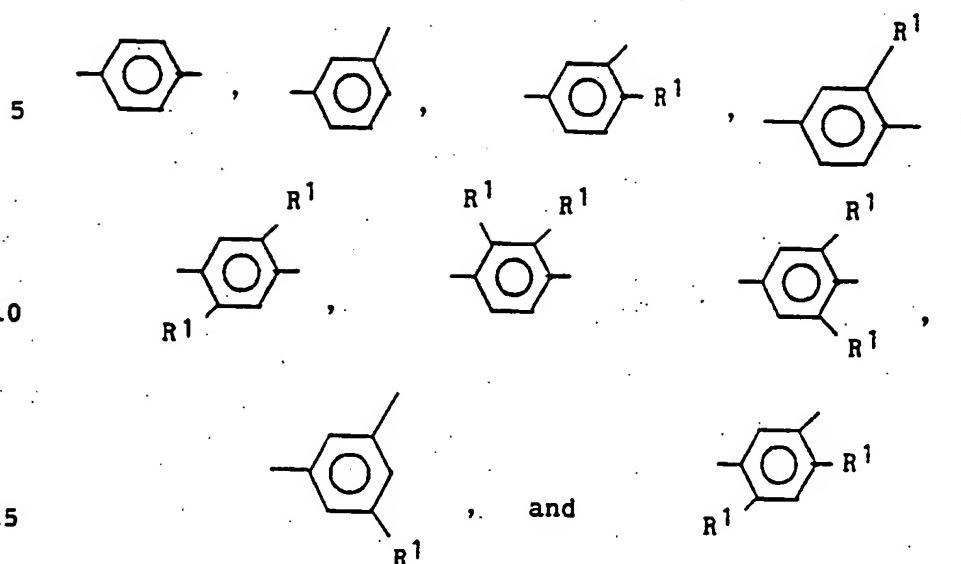
X and X<sup>3</sup> each independently represent either a phenylene group or the formulae

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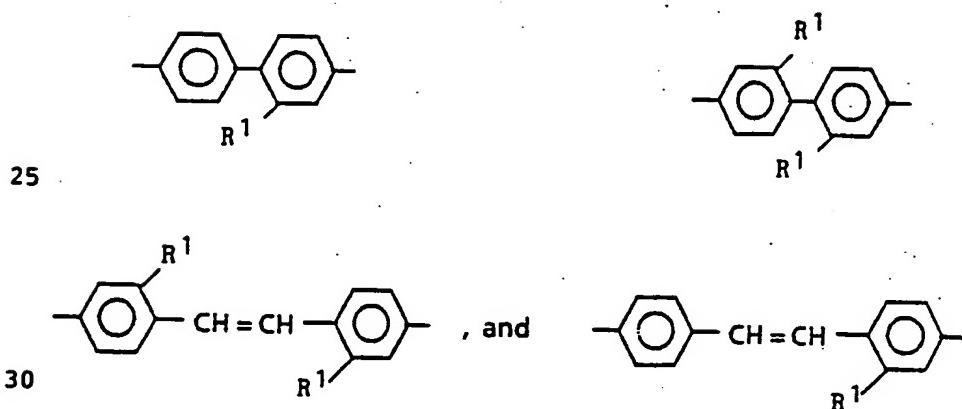
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or a biphenylene group of the formula

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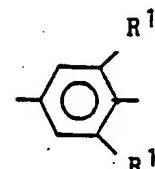
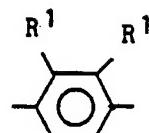
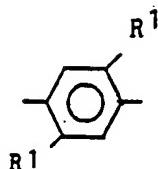
with the proviso that in a compound of formula 1a at least one of X and X<sup>3</sup> must be a biphenylene moiety;

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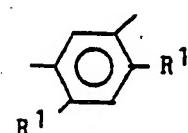
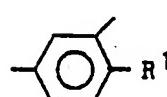
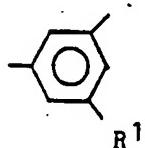
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X" is a group of the formulae

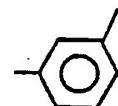
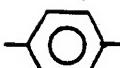
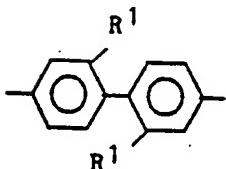
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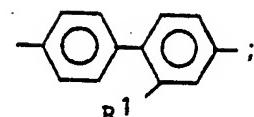
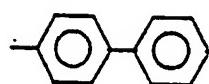
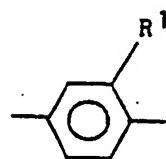


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m is an integer 0 or 1, with the proviso that in a compound of formula 1b when m is 0, R is a hydrogen atom;

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X' can be selected from any of the phenyl or biphenyl groups of X and X³;

-5-

n is an integer of from 3 to 50;

5 R represents a hydrogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl group, a phenyl group, or a phenyl group optionally substituted with 1 or 2 substituents selected from -SO<sub>3</sub>R<sup>2</sup>, -CO<sub>2</sub>R<sup>2</sup>, -PO<sub>3</sub>(R<sup>2</sup>)<sub>2</sub>, or -OPO<sub>3</sub>R<sup>2</sup> and optionally substituted with from 1 to 3 substituents selected from chloro, bromo, or C<sub>1</sub>-C<sub>4</sub> alkyl;

10 R<sup>1</sup> represents -SO<sub>3</sub>R<sup>2</sup>, -CO<sub>2</sub>R<sup>2</sup>, -PO<sub>3</sub>(R<sup>2</sup>)<sub>2</sub>, or -OPO<sub>3</sub>R<sup>2</sup>.

15 R<sup>2</sup> represents a hydrogen atom or a pharmaceutically acceptable cation;

15 R<sup>3</sup> represents -R or -X"-NH<sub>2</sub>, where R and X" are defined as before;

20 R<sup>6</sup> represents H<sub>2</sub>N-X"-NH-, R<sup>2</sup>O-, RNH-, or R-C(=O)-NH-X"-NH-; and

R<sup>7</sup> represents a hydrogen atom, R<sup>2</sup>O-C(=O)-X"-C(=O)-, R-C(=O)-, or RNH-C(=O)-X"-C(=O)-.

25 DETAILED DESCRIPTION OF THE INVENTION

The oligomers of the present invention are polyamides and polyureas having a number average molecular weight Mn of <10,000 comprising recurring units coupled by carbonyl 30 linking moieties, said oligomer having anionic groups and predominantly linear geometry such that regular spacing between anionic groups exists in an aqueous medium. The oligomers are preferably linear in their backbone and also may be in their salt form. Particularly preferred salts 35 are those that are pharmaceutically acceptable.

- The term "pharmaceutically acceptable cation" means a cation acceptable for pharmaceutical use. Those cations that are not substantially toxic at the dosage administered
- 5 to achieve the desired effect and do not independently possess significant pharmacological activity are included within the term "pharmaceutically acceptable cation". Illustratively, these salts include those of alkali metals, such as sodium and potassium; alkaline earth metals, such
- 10 as calcium and magnesium; ammonium; light metals of Group IIIA including aluminum; and organic primary, secondary and tertiary amines, such as trialkylamines, including triethylamine, procaine, dibenzylamine, N,N'-dibenzylethylene-diamine, dihydroabietylamine, N-(C<sub>1</sub>-C<sub>4</sub>)alkylpiperidine,
- 15 and any other suitable amine. Sodium and potassium salts are preferred. The term "pharmaceutically acceptable" means suitable for administration to warm blooded animals, especially human beings, and includes being nontoxic, e.g., suitable for pharmaceutical use and is not poisonous to the
- 20 warm blooded animal. The pharmaceutically acceptable cations of the oligomers of the present invention are prepared by conventional ion exchange processes or by treating the R<sup>1</sup> acid with an appropriate base.
- 25 The oligomers of the present invention are low molecular weight, rigid backbone, water soluble polymers. Additionally, the oligomers have ordered anion spacing. By "ordered anion spacing" or "regular spacing between anionic groups" is meant that the anionic groups (R<sup>1</sup>) are present in
- 30 the backbone of the polymer at intervals determined by the starting material reagent used and the occurrence of the anionic groups is controlled in a predictable manner.
- The terms "predominantly linear geometry" in an aqueous medium refers to the solution configuration of the

-7-

oligomer. A method well known in the art for characterization of the solution configuration of polymer molecules is based on the following formula, referred to as the Mark-Houwink equation ["Introduction to Physical

- 5 Polymer Science", ed. L.H. Sperling, pub. John Wiley & Sons (1985), pp. 81-83],

$$[\eta] = KM^\alpha$$

wherein  $\eta$  is intrinsic viscosity; M is weight average  
10 molecular weight; K is a constant related to chain bond dimension; and  $\alpha$  is a constant determined by polymer configuration. The intrinsic viscosity ( $\eta$ ) for a random coil polymer is  $0.5 < \alpha < 0.9$ ; and for a linear polymer is  $0.98 \leq \alpha < 1.8$ . This formula relates the solution viscosity  
15 "n" to the molecular weight "M". For this invention linear polymers are defined as having " $\alpha$ " values greater than or equal to 0.9. For a rigid rod polymer the theoretical upper limit is 1.8. For a given molecular weight, a higher solution viscosity will be obtained from polymers with a  
20 linear configuration relative to those polymers which exist as a random coil. An additional consideration is that the " $\alpha$ " value is a function of the solvent used. The " $\alpha$ " for a given water soluble polymer may be different at different salt concentrations. For this invention, the salt  
25 concentration is set at the levels present in serum (approximately 80 g/L NaCl, 4 g/L KCl).

As used herein, the term "oligomer" encompasses all the possible values for n, e.g., 3 through 50. The oligomers  
30 are preferably linear with n equal to an integer from 3 to 50, preferably from 3 to 20, more preferably from 3 to 15. Of course, the n value is directly related to the molecular weight of the resulting oligomer. It is essential that these oligomers are of sufficiently low molecular weight in  
35 order to pass through the renal excretory membrane, but

-8-

able to inhibit smooth muscle cell proliferation. The average molecular weight is governed by the stoichiometry of the reagents. The number average molecular weight ( $M_n$ ) is <10,000, preferably from about 400 to about 10,000, and 5 most preferably from about 1,000 to about 6,000.

For the purpose of the present invention, the oligomers described herein and physiologically acceptable salts thereof are considered equivalent. Physiologically 10 acceptable salts refer to the salts of those bases which will form a salt with at least one acid group of the  $R^1$  group and which will not cause significant adverse physiological effects when administered as described herein. Suitable bases include, for example, the alkali 15 metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate and the like, ammonia, primary, secondary and tertiary amines and the like.

20 As for all generic groups of chemical compounds having pharmacological and therapeutic activity, some compounds and subgroups of compounds are preferred. Of those compounds of formulae 1a and 1b, those compounds wherein  $n$  is an integer of from 3 to 18 are preferred, with the 25 compounds wherein  $n$  is an integer of from 6 to 15 being more preferred, and those compounds wherein  $n$  is the integer 9 being most preferred. Also of the compounds of formulae 1a and 1b, those compounds wherein the  $R^1$  groups 30 are a  $-SO_3R^2$ , especially those wherein the  $R^2$  group is a pharmaceutically acceptable cation, most especially those wherein the cation is a sodium cation, are preferred.

-9-

Also preferred are those polyamide compounds of formula la wherein

R<sup>6</sup> is an R-C(=O)-NH-X"-NH- group, especially wherein R is an optionally substituted phenyl group, most especially those wherein R is a phenyl group or a 4-methylphenyl group and wherein X" is a group of one of the formula;

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X<sup>3</sup> is a phenylene group, especially a paraphenylene group of the formula;

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X is a biphenylene group, especially a biphenylene group of one of the formula and

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-10-

R<sup>7</sup> is an R-C(=O)- group especially wherein R is a phenyl group or a 4-methylphenyl group.

Also preferred are those polyurea compounds of formula 5 lb wherein

R is an optionally substituted phenyl group especially those wherein R is a phenyl group or a 4-methylphenyl group;

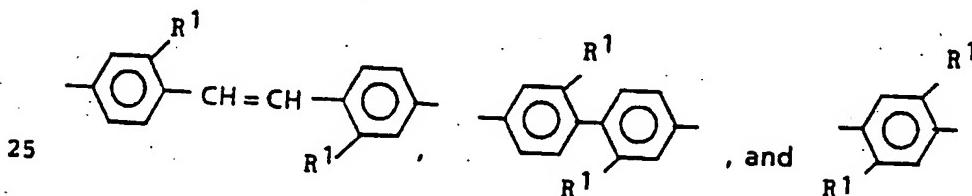
m is the integer 1;

X' is a phenyl or biphenyl group substituted by one or two -SO<sub>3</sub>R<sup>2</sup> groups, especially those wherein R<sup>2</sup> is a sodium cation; and

R<sup>3</sup> is an optionally substituted phenyl group especially those wherein R is a phenyl group or a 4-methylphenyl group.

Particularly preferred are those formula lb compounds wherein X' is a phenyl or biphenyl group of one of the following formulae

20



especially those wherein the R<sup>1</sup> group is a -SO<sub>3</sub>R<sup>2</sup> wherein 30 the R<sup>2</sup> group is a sodium cation.

The oligomers can be prepared by the procedures described in European Patent Application 91111315.7, filed July 8, 1991, published January 22, 1992.

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-11-

The ability of the sulfonated oligomers of this invention to act as inhibitors of smooth muscle cell proliferation can be demonstrated by their ability to suppress DNA replication in cultured rat vascular smooth muscle cells (VSMC) stimulated to proliferate by the addition of serum (Fig. 1, Panel A) or instead, specific growth factors such as platelet derived growth factor (PDGF) or other agents (Fig. 2, Panels A and B, respectively) with known mitogenic activity on VSMC. The inhibitory activity is associated with polymer length being optimal at chain lengths between 3 and 15 and exhibits a dose-dependent inhibition of DNA replication and a corresponding decrease on cell numbers (Fig. 1, Panel B). The correlation between decreased DNA synthesis (Fig. 1, Panel A) and reduced cell numbers (Fig. 1, Panel B) in cultures after 72 hours of exposure to noncytotoxic concentrations of drug shows that these compounds are antiproliferative. In addition, the compounds block the proliferation of rabbit vascular smooth muscle cells (Fig. 3) and calf coronary (Fig. 4) and bovine pulmonary vascular smooth muscle cells (Fig. 5). They are also effective as antiproliferative agents when added as late as 12 hours post-mitogenic stimulation with the known smooth muscle cell mitogen PGDF (Fig. 6).

25

The bioavailability and *in vivo* activity of such compounds are demonstrated in Fig. 7. The experiment employs a rat restenosis model in which arterial denudation by balloon catheterization removes the arterial endothelial cell layer and stimulates VSMC migration and proliferation. A single intraperitoneal injection daily for two days of MDL 100,127 followed arterial denudation. The animals were then sacrificed and the DNA replication rate of the cells within the rat abdominal aorta was determined *ex vivo*. The aortas were removed from the animals, and pulsed as an

-12-

explant with [<sup>3</sup>H]thymidine (a DNA precursor) to measure its incorporation into DNA relative to that of aortas from the sham operated rats as negative controls, and with nontreated but balloon catheterized, animals as positive controls. The data of Figure 7 show that MDL 100,127, when administered to rats, blocked DNA replication of the SMC in the aortic vessel wall of these animals.

The amount of the anionic polyamide and polyurea oligomer of formula 1a or 1b to be administered can vary widely according to the particular dosage unit employed, the period of treatment, the age and sex of the patient treated, the nature and extent of the disorder treated, and the particular anionic polyamide or polyurea of formulae 1a or 1b selected. Moreover the anionic polyamide and polyureas of formulae 1a and 1b can be used in conjunction with other agents useful in inhibiting smooth muscle cell proliferation and agents known to be useful to treat the symptoms of and complications associated with diseases and conditions caused by smooth muscle cell proliferation. The smooth muscle cell inhibitory effective amount of anionic polyamide and polyurea oligomer of formula 1a and 1b to be administered will generally range from about 15 mg/kg to 500 mg/kg. A unit dosage may contain from 25 to 500 mg of anionic polyamide and polyurea of formulae 1a and 1b, and can be taken one or more times per day. The polyamide and polyurea oligomers can be administered with a pharmaceutical carrier using conventional dosage unit forms either orally, parenterally, or topically.

The term "patient" used herein is taken to mean mammals such as primates, including humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice.

-13-

The preferred route of administration is oral administration. For oral administration the anionic polyamide and polyurea oligomers can be formulated into solid or liquid preparations such as capsules, pills, 5 tablets, troches, lozenges, melts, powders, solutions, suspensions, or emulsions. The solid unit dosage forms can be a capsule which can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers such as lactose, sucrose, 10 calcium phosphate, and cornstarch. In another embodiment the compounds of this invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch, or gelatin, disintegrating agents intended to 15 assist the break-up and dissolution of the tablet following administration such as potato starch, alginic acid, corn starch, and guar gum, lubricants intended to improve the flow of tablet granulations and to prevent the adhesion of tablet material to the surfaces of the tablet dies and 20 punches, for example, talc, stearic acid, or magnesium, calcium, or zinc stearate, dyes, coloring agents, and flavoring agents intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient. Suitable excipients for use in oral liquid 25 dosage forms include diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptably surfactant, suspending agent, or emulsifying agent.

30

The anionic polyamide and polyurea oligomers of this invention may also be administered parenterally, that is, subcutaneously, intravenously, intramuscularly, or interperitoneally, as injectable dosages of the compound in 35 a physiologically acceptable diluent with a pharmaceutical

-14-

carrier which can be a sterile liquid or mixture of liquids such as water, saline, aqueous dextrose and related sugar solutions, an alcohol such as ethanol, isopropanol, or hexadecyl alcohol, glycols such as propylene glycol or

5 polyethylene glycol, glycerol ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers such as poly(ethylene-glycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable

10 surfactant such as a soap or a detergent, suspending agent such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agent and other pharmaceutically adjuvants. Illustrative of oils which can be used in the parenteral formulations of

15 this invention are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum, and mineral oil. Suitable fatty acids include oleic acid, stearic acid, and isostearic acid. Suitable

20 fatty acid esters are, for example, ethyl oleate and isopropyl myristate. Suitable soaps include fatty alkali metal, ammonium, and triethanolamine salts and suitable detergents include cationic detergents, for example, dimethyl dialkyl ammonium halides, alkyl pyridinium

25 halides, and alkylamines acetates; anionic detergents, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates; nonionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenopolypropylene

30 copolymers; and amphoteric detergents, for example, alkyl-beta-aminopropionates, and 2-alkylimidazoline quaternary ammonium salts, as well as mixtures. The parenteral compositions of this invention will typically contain from about 0.5 to about 25% by weight of the anionic polyamide

35 and polyurea oligomers of formula 1a and 1b in solution.

-15-

Preservatives and buffers may also be used advantageously. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophile-lipophile balance (HLB) of 5 from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5 to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB. Illustrative of surfactants used 10 in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

15

The compounds of this invention can also be administered topically. This can be accomplished by simply preparing a solution of the compound to be administered, preferably using a solvent known to promote transdermal 20 absorption such as ethanol or dimethyl sulfoxide (DMSO) with or without other excipients. Preferably topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety.

25

Some suitable transdermal devices are described in U.S. Pat. Nos. 3,742,951, 3,797,494, 3,996,934, and 4,031,894. These devices generally contain a backing member which defines one of its face surfaces, an active agent permeable 30 adhesive layer defining the other face surface and at least one reservoir containing the active agent interposed between the face surfaces. Alternatively, the active agent may be contained in a plurality of microcapsules distributed throughout the permeable adhesive layer. In 35 either case, the active agent is delivered continuously

-16-

from the reservoir or microcapsules through a membrane into the active agent permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the active agent is absorbed through the skin, a controlled and  
5 predetermined flow of the active agent is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

In another device for transdermally administering the  
10 compounds in accordance with the present invention, the pharmaceutically active compound is contained in a matrix from which it is delivered in the desired gradual, constant and controlled rate. The matrix is permeable to the release of the compound through diffusion or microporous  
15 flow. The release is rate controlling. Such a system, which requires no membrane is described in U.S. Pat. No. 3,921,636. At least two types of release are possible in these systems. Release by diffusion occurs when the matrix is non-porous. The pharmaceutically effective compound  
20 dissolves in and diffuses through the matrix itself. Release by microporous flow occurs when the pharmaceutically effective compound is transported through a liquid phase in the pores of the matrix.

25 Definitions

The terms used in the present application are defined as follows:

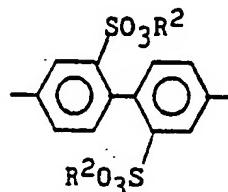
30 n represents the number average repeat length of the distribution through all formulae.

MDL 101,758, 100,127, and 101,044 mean poly{imino[2,2'-disulfo(1,1'-biphenyl)-4,4'-diyl]iminocarbonyl}, alpha-[(4-methylphenyl)amino]-carbonyl}-omega-[(4-methylphenyl)amino]-  
35

-17-

and is represented by Formula 1b above when R is 4-methylphenyl, R<sup>2</sup> is sodium, X is

5



10 and n is 3, 6, and 9, respectively.

#### EXAMPLES

The following examples illustrate various aspects of  
15 the present invention:

##### EXAMPLE 1

20 TAB. INHIBITION OF VASCULAR SMOOTH MUSCLE CELL  
PROLIFERATION BY POLYMERS

Stimulus	Treatment†	IC <sub>50</sub> (μg/ml)		
		MDL 101,044 (n = 9)	MDL 100,127 (N = 6)	MDL 100,758 (n = 3)
PDGF (5ng/ml)	post (24 hours) pre (24 hours)	1.10 1.23	1.08 0.27	2.39 0.46
TPA (50nM)	pre (24 hours)	2.27	2.44	3.12
FBS (10%)	post (24 hours) pre (24 hours) + post (21 hours)	>28.4 9.64	>40.7 10.60	>68.8 17.90

25 † post; length of incubation time with compound after VSMC stimulation;  
30 pre; length of incubation time with compound prior to VSMC stimulation.

-18-

EXAMPLE 2

SMOOTH MUSCLE CELL PROLIFERATION ASSAYS

5 Cell Culture

Vascular smooth muscle cells (SMC) were isolated enzymatically from 2-3 thoracic aortas of male Sprague-Dawley rats (150 g). Briefly, aseptically obtained aortic strips were pre-digested at 37°C in serum-free Dulbecco's Modified Eagle's Medium (DMEM) containing collagenase (1 mg/ml) and elastase (0.5 mg/ml) for 15-30 min. The luminal side of the aortic strip was gently scraped to remove the endothelium and the medial layer was separated from the adventitia. The medial layer was rinsed and digested for an additional 2 hours in fresh enzyme mixture at 37°C. Cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) at 37°C with 5% CO<sub>2</sub> in humidified air. Subculture was performed every 7 days after the culture had reached confluence. Cultures between the 5th and 9th passages were employed in the present study.

25 <sup>3</sup>H-thymidine incorporation

SMC were removed from their culture flasks by trypsinization and seeded at equal density (5.0 x 10<sup>4</sup>/well) into 24-well tissue culture plates. Cells were allowed to attach and grow to a near-confluent state in DMEM and 10% FBS (growth medium). This growth medium was then removed and replaced with DMEM and 0.2% FBS, and cells were incubated for 48-72 hours to achieve a growth arrest. Quiescent cells were then incubated for 21 h in DMEM and mitogens or growth factors (TDA, PDGF or FBS), containing various concentrations of compounds. In some experiments,

-19-

quiescent cells were treated with various concentrations of compounds for 24 hours, washed twice with DPBS and incubated for 21 h in DMEM and growth factors. Finally, cells were incubated for 1-3 h in freshly prepared DMEM 5 containing 1 $\mu$ CI [3H-methyl]thymidine to measure DNA synthesis. Experiments were terminated by washing cells with ice cold PBS, precipitation of acid-insoluble material with ice-cold 10% trichloroacetic acid (TCA) and extraction of DNA with 1N NaOH. After neutralization with 5N HCl, the 10 contents of each well was added to a scintillation vial with scintillation cocktail and counted in a liquid scintillation counter.

Cell growth

15 SMC were seeded into 12-well tissue culture plates at the density of 10<sup>5</sup> cells/well. Cells were allowed to attach and grow for 3 days in growth medium. Medium was then replaced with fresh growth medium containing various concentrations of compounds (day 0) and incubated for 3 20 days. Cell numbers were counted on day 0 and day 3 in a Coulter counter.

Using these procedures, the effects of the oligomers of this invention on smooth muscle cell proliferation were 25 determined and the results are indicated in Figures 1 through 7.

**EXPLANATION OF FIGURES**

30 Figure 1 shows the dose-dependent inhibition of serum-induced rat vsmc (rvsmc) proliferation by three polyamide biphenyl disulfonic acid phthalate copolymers, MDL 101,044 (n=9), 100,127 (n=6), and 100,758 (n=3). Panel A: Effect 35 of compounds on DNA replication. 5 x 10<sup>4</sup> rvsmc were synchronized into the G<sub>0</sub> state by a 48 h preincubation in

-20-

serum-deprived media containing 0.2% fetal bovine serum (FBS). Cells were then switched to Dulbecco's minimal essential media (DMEM) containing various concentrations of drug for 24 h. To stimulate proliferation FBS was added to 5 a final concentration of 10% and the cells were incubated for 22 h. The medium was then replaced with DMEM containing 1 $\mu$ Ci of  $^3$ H-thymidine and pulse-labeled for 1 h. Cells were then washed 2 times with PBS, solubilized in 1 M NaOH and counted. Panel B: Effect of compounds on cell 10 numbers. Cells were seeded at  $5.18 \times 10^4$  cells/well in DMEM + 10% FBS containing various concentrations of compounds. Cells were incubated 3 days and then cell numbers counted. The results were normalized to cell cultures not receiving compound (control) and expressed as % of control. 15 Viability was determined by trypan blue exclusion.

Figure 2 Panel A shows the effect of the various polymers on PDGF-induced DNA replication as a measure of inhibition of rvsmc cell proliferation. Cells ( $5 \times 10^4$ ) 20 were growth-arrested in 0.2% FBS for 2 days to synchronize the cultures. Cells were washed twice with PBS and then treated with various concentrations of the indicated drugs in DMEM for 24 h. Cells were washed twice in PBS and then incubated in DMEM containing 1 $\mu$ Ci/well  $^3$ H-thymidine. 25 Proliferation was determined as in Fig. 1. Panel B: Effect of various polymers on phorbol ester (TPA)-induced rvsmc proliferation. Cells were seeded, treated with compounds and pulse-labeled with  $^3$ H-thymidine as performed in Panel A above.

30

Figure 3 shows the inhibition of PDGF-induced proliferation of rabbit vascular smooth muscle cells by MDL 100,127. Numbers shown represent the [MDL 100,127] in  $\mu$ g/ml. Cells ( $5 \times 10^4$ ) were serum-arrested in 0.2% FBS for 35 24 h, stimulated with PDGF in 0.2% horse plasma-derived

-21-

serum (PDS) containing the indicated MDL 100,127 concentrations for 21 h and then pulse-labeled for 1 h with 1 $\mu$ Ci  $^3$ H-thymidine/well. Total counts/well were determined after solubilization in 1 M NaOH. DMEM: cells receiving  
5 DMEM only; PDGF: cells stimulated with 5 ng/ml PDGF; and cells stimulated with 5 ng/ml PDGF in 50, 10, 2, 0.4 and 0.08  $\mu$ g/ml MDL 100,127.

Figure 4 shows the inhibition of PDGF-induced  
10 proliferation of calf coronary vsmc by MDL 100,127. Conditions and procedures are identical to those in Fig. 3.

Figure 5 shows the inhibition of PDGF-induced proliferation of calf pulmonary vascular smooth muscle  
15 cells by MDL 100,127. Conditions and procedures are identical to those of Fig. 3.

Figure 6 shows the effect of 20  $\mu$ g/ml MDL 100,127 on rvsme proliferation when added at various times post-  
20 stimulation of cells with 5 ng/ml PDGF. Cells were synchronized for 48 h in 0.2% FBS and stimulated by 5 ng/ml PDGF in 0.2% PDS. MDL 100,127 was added either at the time of PDGF stimulation (time 0) or at various times post-stimulation with PDGF. Cultures were pulse-labeled for 2 h  
25 with 1 $\mu$ Ci  $^3$ H-thymidine/well beginning at 21 h post-stimulation with PDGF in all of the experiments performed. The cells were then harvested and counted. Parallel samples to which MDL 100,127 was added 15 h and 18 h post-PDGF stimulation were used to determine the amount of  
30 trichloroacetic acid (TCA) soluble and TCA insoluble counts present in total cellular soluble thymidine pools and DNA, respectively. The 15 h and 18 h samples were incubated for 6 and 3 h, respectively, in 20  $\mu$ g/ml MDL 100,127 before the pulse label. The presence of these compounds did not block  
35 uptake of  $^3$ H-thymidine in soluble cellular pools or in DNA

-22-

at these two time points. These findings show that once the rvsme are committed to S-phase of the cell cycle the compounds do not block the uptake of thymidine. Thus, the antiproliferative block occurs prior to the commitment to 5 S-phase and does not reflect a non-specific inhibition of uptake of thymidine by the compound.

Figure 7 shows that MDL 100,127 blocks the smooth muscle proliferative response *in vivo* in rats subjected to 10 aortic balloon catheterization. The experimental protocol was a modification of the original aortic balloon injury model described by Baumgartner et al., Ges. Exp. Med 137, p. 227 (1963). Male Sprague-Dawley rats (160-190 g) were lightly anesthetized with 50 mg/kg, i.p. sodium thiamylol 15 (Parke-Davis, Morris Pains, NJ). A Fogarty embolectomy balloon (catheter size 2F, Baxter) was inserted by way of the carotid artery and passed down to the abdominal artery. The catheter was withdrawn three times to induce injury with the balloon distended with 100% CO<sub>2</sub> (23 psi). The 20 balloon was then deflated, the catheter removed, the animals sutured and allowed to recover. The positive control group (n=6) were balloon catheterized and did not receive drug. The experimental group (n=7) were also catheterized but received 30 mg/kg i.p. of MDL 100,127 at 25 the time of balloon injury and at 24 h post-injury. The negative control group (n=6) were sham operated and did not receive drug. These animals received surgery and were treated identically to the positive control group; they were not balloon catheterized. All animals survived the 30 procedure and were sacrificed 48 h post-injury. Aortas were removed, adipose tissue was removed, the aortas explanted into DMEM containing 10 µCi 3H-thymidine and pulse-labeled for 1 hour. The tissues were then washed 2 times in PBS, the DNA extracted by standard procedures and 35 counted. All animals receiving balloon catheterization

-23-

showed visible signs of aortic injury of swelling and clot formation.

As is shown in Fig. 7, administration of MDL 100,127 significantly suppressed aortic smooth muscle cell proliferation as evidenced by the inhibition of  $^3\text{H}$ -thymidine into DNA relative to the positive control group ( $p<0.01$ ). Slightly higher counts were obtained in the treated group relative to the sham operated group ( $p<0.05$ ).

10

Table 1 shows that MDL 101,044 and 100,127 have minimal effects on the coagulation of bovine plasma relative to the potent anticoagulant heparin. The prothrombin (PT) and activated partial thromboplastin times (APTT) in seconds were only mildly elevated at the highest compound concentrations.

20

TABLE 1  
EFFECT OF COMPOUNDS ON THE COAGULATION  
PROPERTIES OF BOVINE PLASMA

[Compound] μg/ml	Heparin		MDL 101,044c	
	APTT <sup>a</sup>	PT <sup>a</sup>	APTT	PT
25	94	20	94 (92)	20 (20)
3.3	161	24	90 (92)	20 (20)
10.0	NCD <sup>b</sup>	58	91 (88)	21 (21)
16.7	NCD	91	97 (90)	22 (21)
30	23.3	NCD	120 (102)	23 (22)
35	30.0	NCD	NCD (109)	25 (23)

a APTT/PT, activated partial thromboplastin/prothrombin times measured in seconds

b NCD, no clot detected

c numbers in parenthesis represent MDL 100,127 (n=6)

-24-

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed

5 herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

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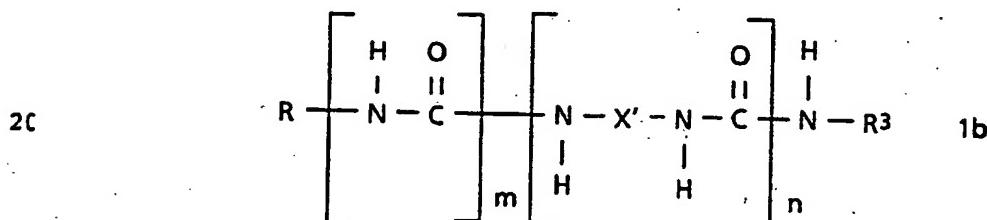
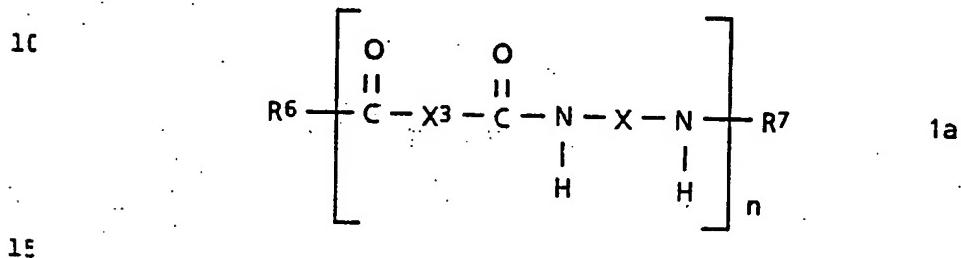
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## WHAT IS CLAIMED IS:

1. A method of inhibiting proliferation of smooth muscle cells in a patient in need thereof which comprises  
 5 administering to the patient an antiproliferative amount of an anionic polyamide and polyurea oligomer of formulae 1a and 1b, respectively,



25

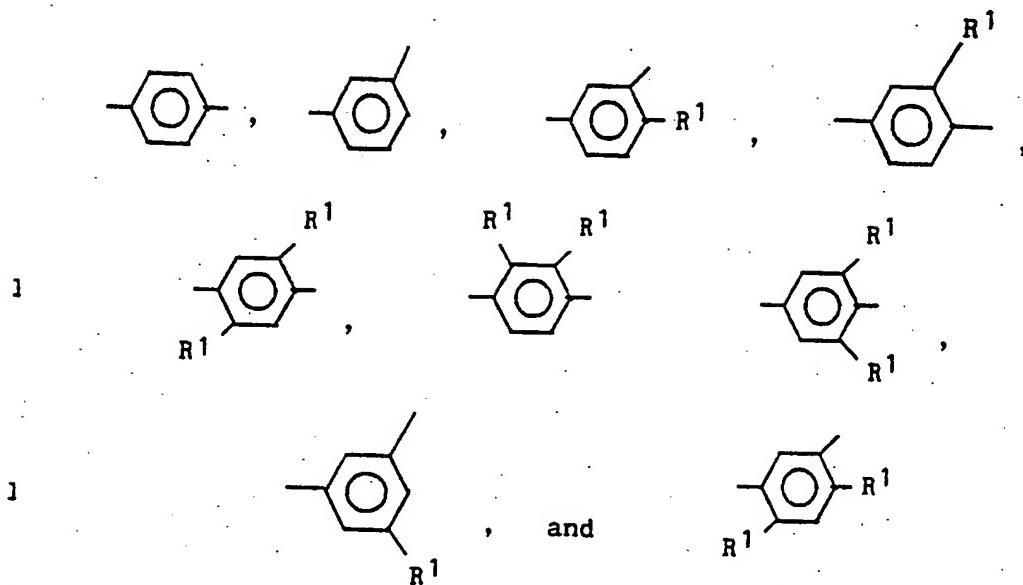
wherein

X and X<sup>3</sup> each independently represent either a phenylene group of the formulae

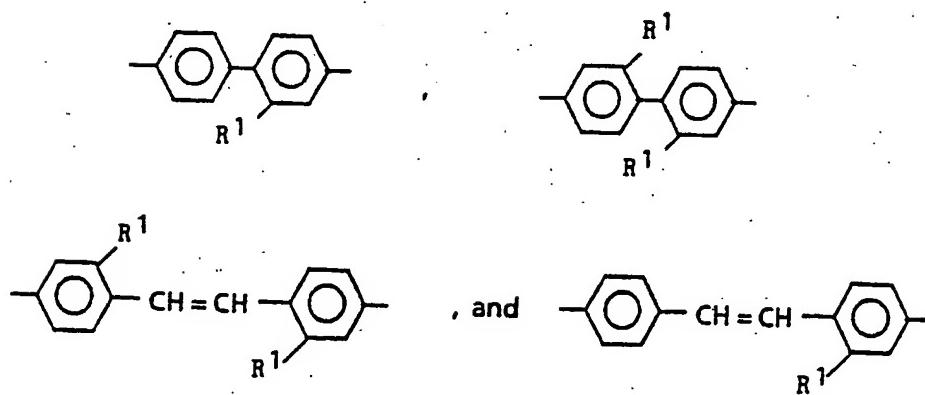
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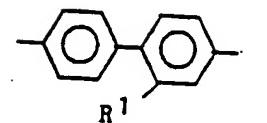
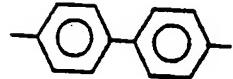
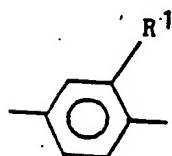
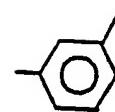
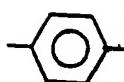
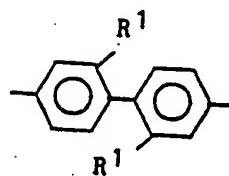
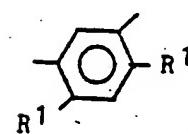
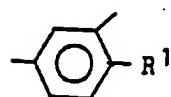
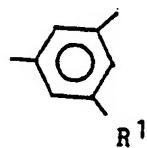
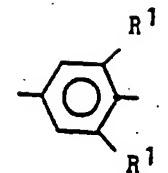
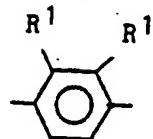
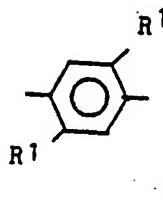
or a biphenylene group of the formulae



with the proviso that in a compound of formula 1a at least one of X and X<sup>3</sup> must be a biphenylene moiety;

-27-

$X''$  is a group of the formulae



30

$m$  is an integer 0 or 1, with the proviso that in a compound of formula 1b when  $m$  is 0, R is a hydrogen atom;

35

$X'$  can be selected from any of the phenyl or biphenyl groups of X and  $X_3$ ;

-28-

n is an integer of from 3 to 50;

5 R represents a hydrogen atom, a C<sub>1</sub>-C<sub>4</sub> alkyl group, a phenyl group, or a phenyl group optionally substituted with 1 or 2 substituents selected from -SO<sub>3</sub>R<sup>2</sup>, -CO<sub>2</sub>R<sup>2</sup>, -PO<sub>3</sub>(R<sup>2</sup>)<sub>2</sub>, or -OPO<sub>3</sub>R<sup>2</sup> and optionally substituted with from 1 to 3 substituents selected from chloro, bromo, or C<sub>1</sub>-C<sub>4</sub> alkyl;

10

R<sup>1</sup> represents -SO<sub>3</sub>R<sup>2</sup>, -CO<sub>2</sub>R<sup>2</sup>, -PO<sub>3</sub>(R<sup>2</sup>)<sub>2</sub>, or -OPO<sub>3</sub>R<sup>2</sup>;

15

R<sup>2</sup> represents a hydrogen atom or a pharmaceutically acceptable cation;

15

R<sup>3</sup> represents -R or -X"-NH<sub>2</sub>, where R and X" are defined as before;

20

R<sup>6</sup> represents H<sub>2</sub>N-X"-NH-, R<sup>2</sup>O-, RNH-, or R-C(=O)-NH-X"-NH-; and

R<sup>7</sup> represents a hydrogen atom, R<sup>2</sup>O-C(=O)-X"-C(=O)-, R-C(=O)-, or RNH-C(=O)-X"-C(=O)-.

25

2. A method of claim 1 wherein n is an integer of from 6 to 15.

30

3. A method of claim 1 wherein n is the integer 9.

4. A method of claim 2 wherein R<sup>1</sup> is a -SO<sub>3</sub>R<sup>2</sup> group.

5. A method of claim 4 wherein R<sup>2</sup> is a sodium cation.

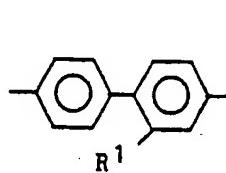
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6. A method of claim 4 wherein R<sup>6</sup> is a R-C(=O)-NH-X"-NH- group.

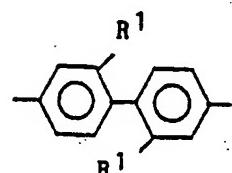
-29-

7. A method of claim 6 wherein R is a phenyl or a 4-methylphenyl group.

8. A method of claim 6 wherein X<sup>n</sup> is a group of the 5 formulae



and

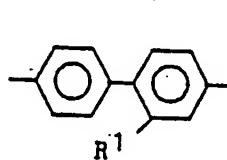


9. A method of claim 4 wherein R<sup>7</sup> is a R-C(=O)- group.

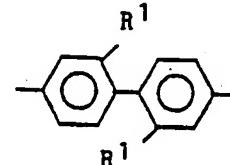
10. A method of claim 9 wherein R is a phenyl or a 15 4-methylphenyl group.

11. A method of claim 4 wherein X<sup>3</sup> is a paraphenylene group.

20 12. A method of claim 4 wherein X is a group of the formula



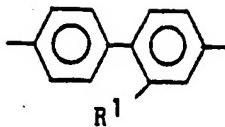
and



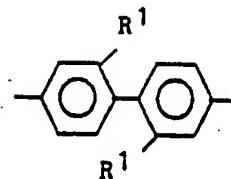
30 13. A method of claim 4 wherein R<sup>6</sup> is a R-C(=O)-NH-X"-NH-, X<sup>3</sup> is a paraphenylene group, X is a biphenylene group of the formulae

-30-

5



and

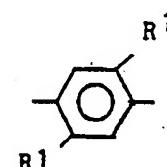
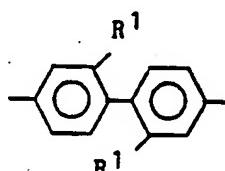
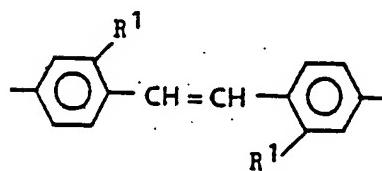


R<sup>7</sup> is a R-C(=O)- group, and R is a 4-methylphenyl group.

14. A method of claim 4 wherein m is the integer 1.  
10

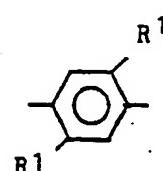
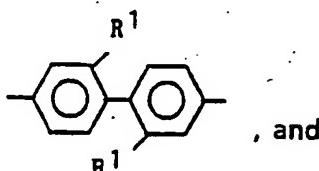
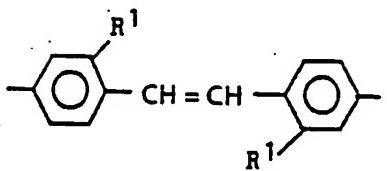
15. A method of claim 14 wherein R is a phenyl or  
4-methylphenyl group.

16. A method of claim 14 wherein X' is a group of one  
15 of the formulae



, and

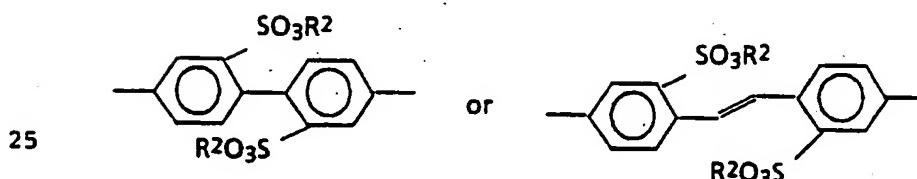
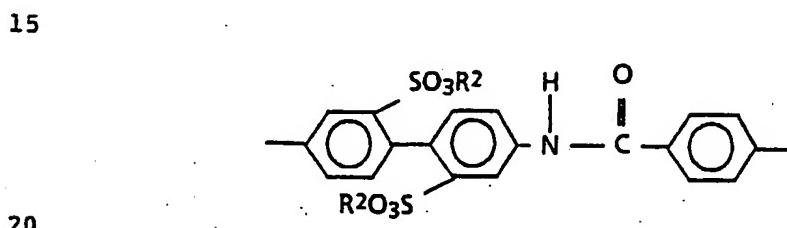
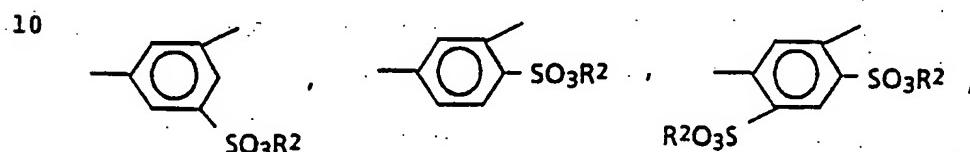
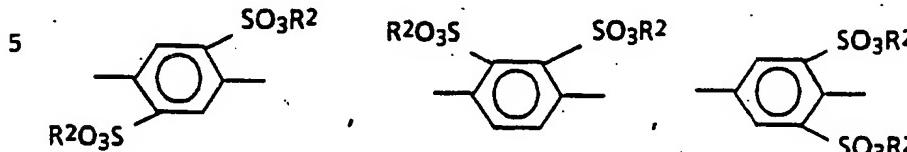
17. A method of claim 14 wherein R is a 4-methylphenyl  
group, X' is a group of the formulae



and R<sup>3</sup> is a 4-methylphenyl group.

-31-

18. A method of claim 1 wherein the oligomer is a polyurea of Formula I wherein R and R<sup>3</sup> are a 4-methylphenyl group; m is 1; n is 3 to 15; X represents



and  $R^2$  is as defined as in claim 1.

30 19. The use of the compounds of claim 1 for the manufacture of pharmaceutical compositions useful in the inhibition of smooth muscle cell proliferation.

FIG.1a

1/7

## Effect of Polymers on Serum- Induced SMC Proliferation

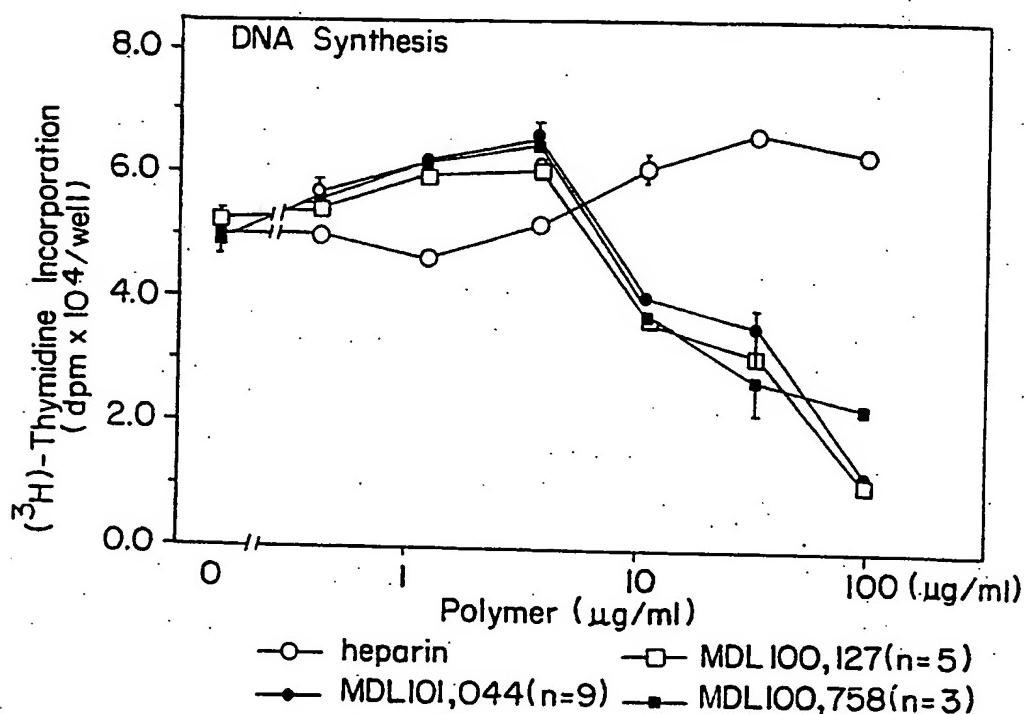


FIG.1b Effect of Polymers on Serum- Induced SMC Proliferation

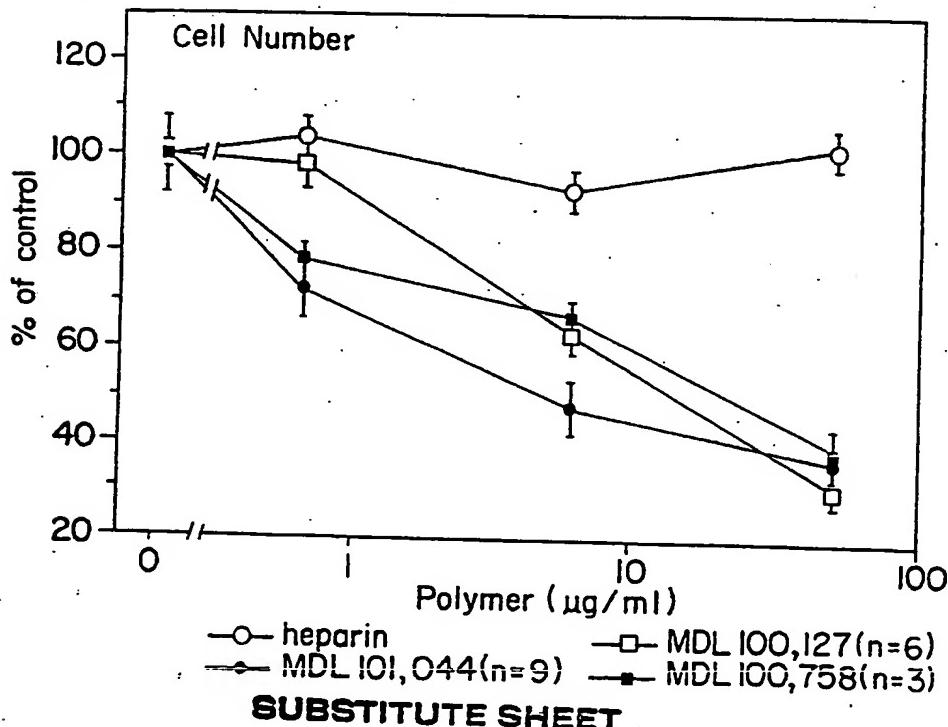
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FIG. 2a

2/7

## Effect of Polymers on DNA Synthesis in SMC

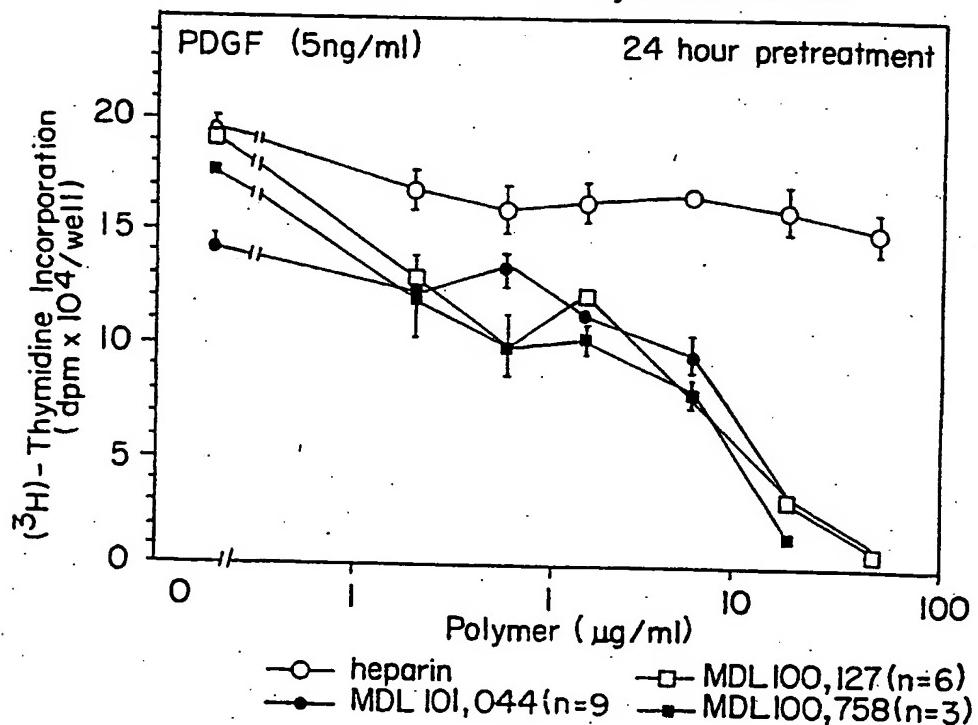
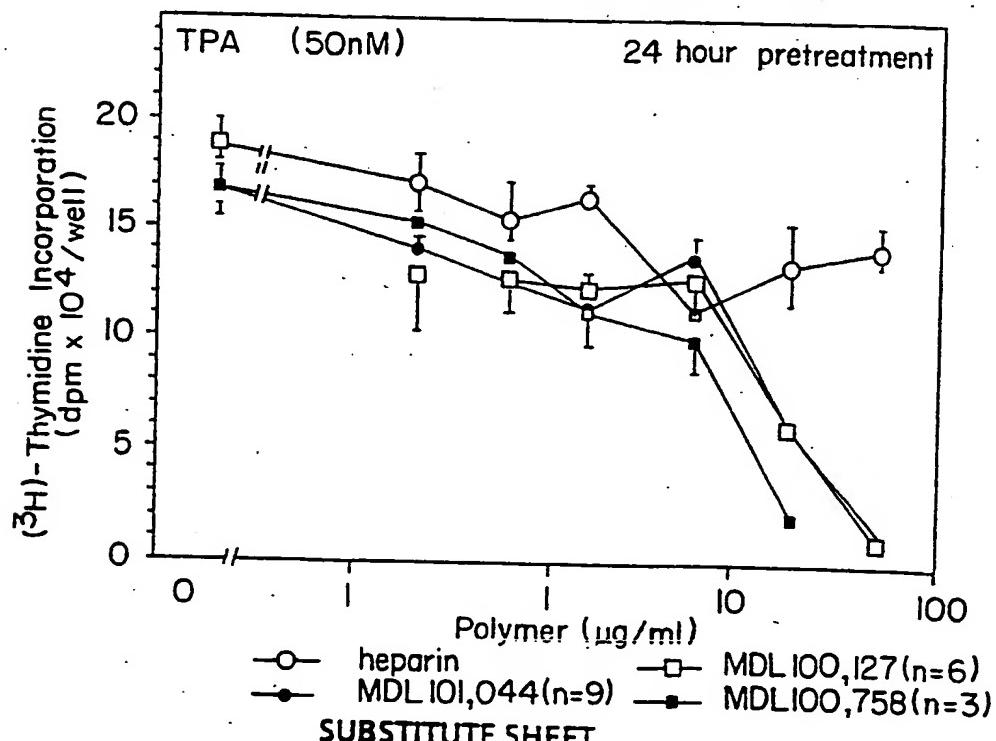


FIG. 2b

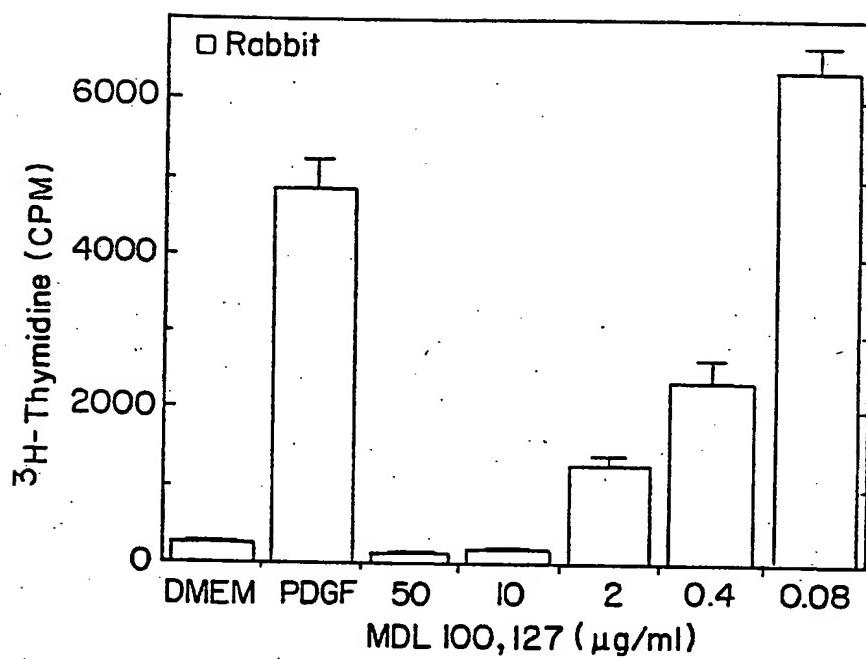
## Effect of Polymers on DNA Synthesis in SMC



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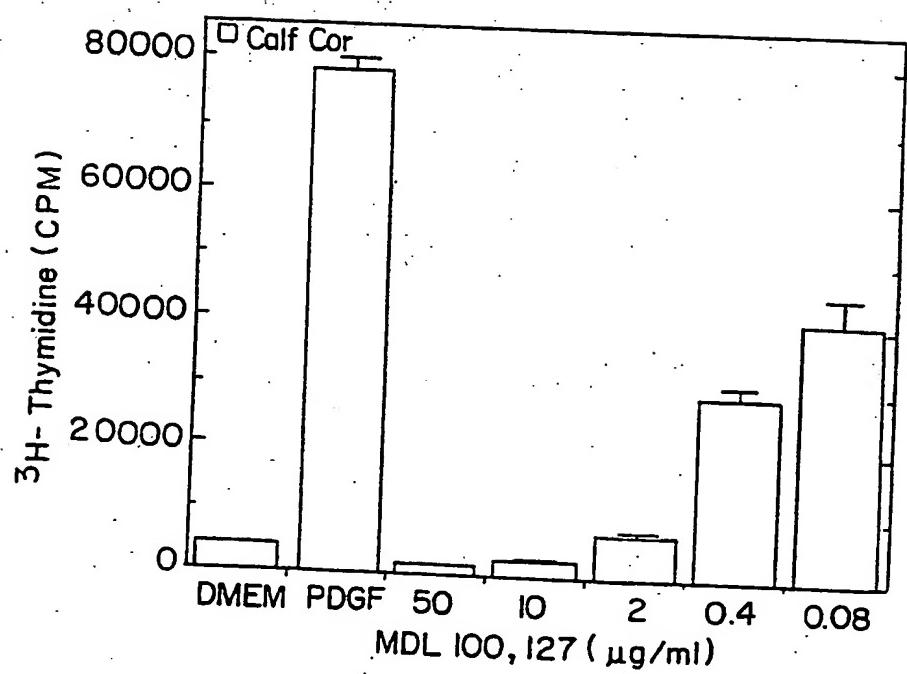
FIG. 3



SUBSTITUTE SHEET

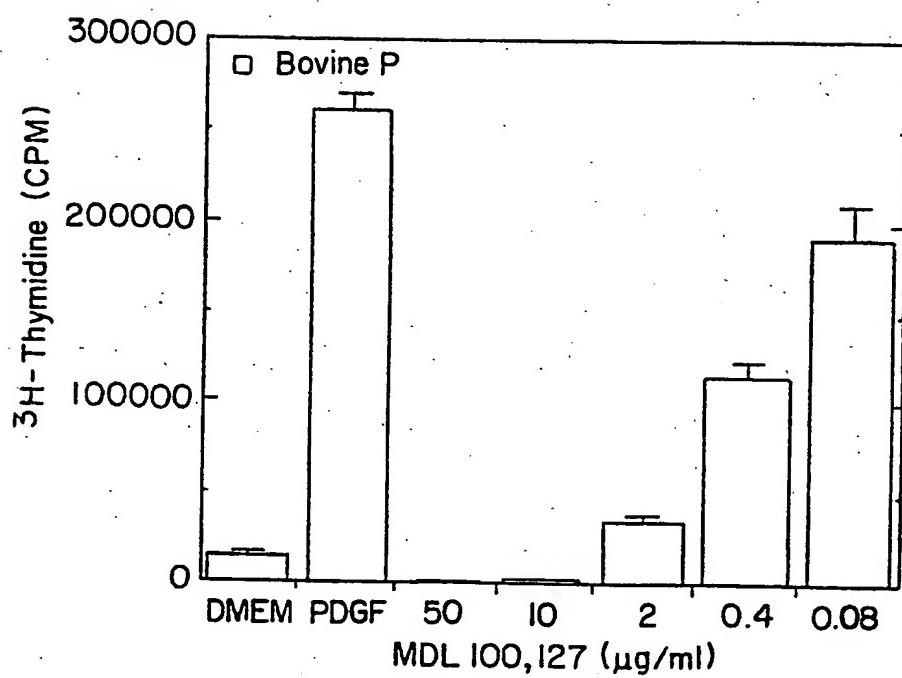
4/7

FIG. 4



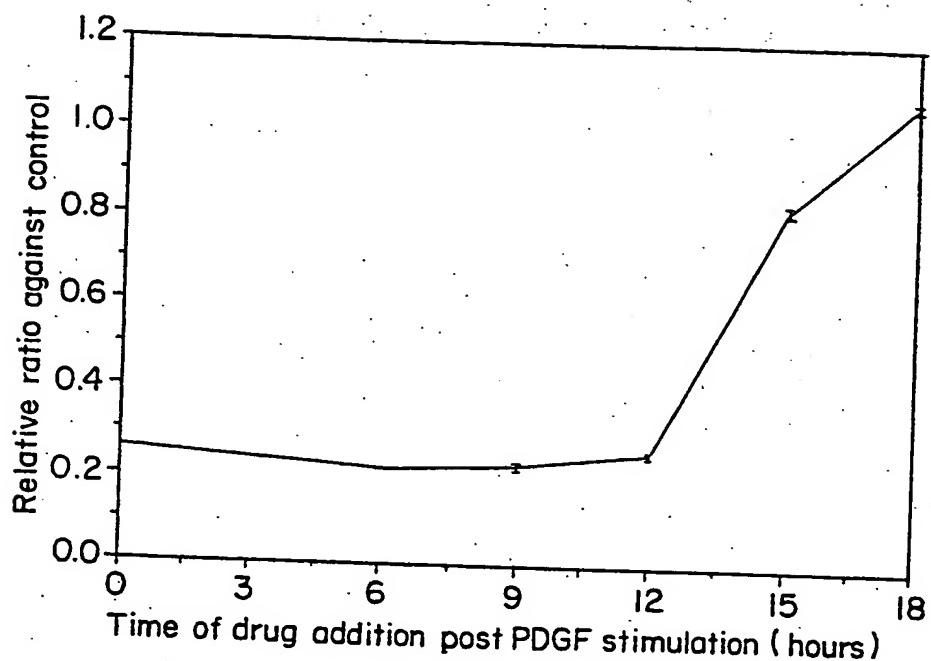
5/7

FIG. 5



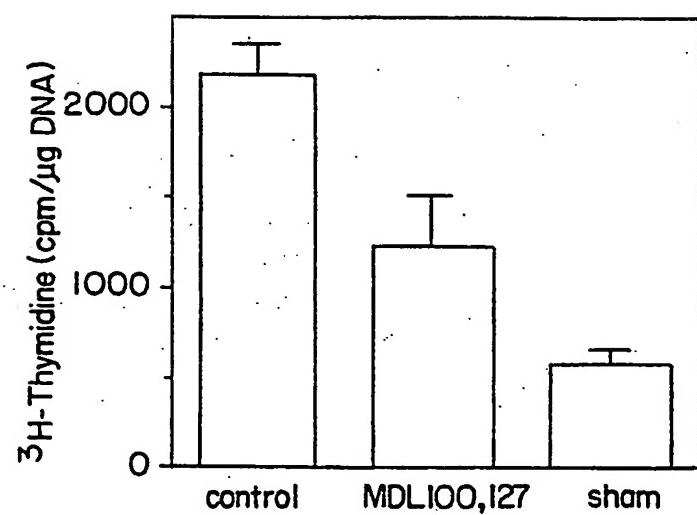
6/7

FIG.6



7/7

FIG. 7



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 93/06693

## I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)<sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K31/795; A61K31/185; A61K31/785; A61K31/80

## II. FIELDS SEARCHED

Minimum Documentation Searched<sup>7</sup>

Classification Symbols

Classification System	A61K
Int.Cl. 5	

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched<sup>8</sup>

## III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup>

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	<p>EP,A,0 467 185 (THE DOW CHEMICAL COMPANY)            22 January 1992            cited in the application            see abstract            see page 2, line 42 - line 50            see page 3, line 27 - line 29; claims;            examples 1-3,8            see examples I-VII</p> <p>---</p>	1-19
P,Y	<p>J. CELL BIOCHEM.            vol. SUPPL, January 1993,            TYMS ET AL., CARDIN ET AL. 'pages 365,382            and 383'            see the whole document</p> <p>---</p>	1-19 -/-

\* Special categories of cited documents :<sup>10</sup>

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
- "C" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "D" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

05 OCTOBER 1993

Date of Mailing of this International Search Report

14. 10. 93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

HOFF P.J.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	<p>NATURE vol. 265, no. 5595, 1977, pages 625 - 626</p> <p>A.W. CLOWES ET AL. 'SUPPRESSION BY HEPARIN OF SMOOTH MUSCLE CELL PROLIFERATION IN INJURED ARTERIES' see the whole document</p> <p>---</p>	1-19
Y	<p>JOURNAL OF VASCULAR SURGERY vol. 10, no. 5, 1989, pages 589 - 591</p> <p>A.W. CLOWES ET AL. 'INHIBITION BY HEPARIN OF SMOOTH MUSCLE HYPERPLASIA' see the whole document</p> <p>---</p>	1-19
A	<p>WO,A,9 103 226 (RORER INTERNATIONAL) 21 March 1991 see abstract see page 2, line 9 - page 4, line 5 see page 7, line 20 - page 8, line 25 see page 11, line 30 - line 32; claims 1-10; example 6</p> <p>---</p>	1-19
A	<p>GB,A,781 479 (CIBA LIMITED) 21 August 1957 see the whole document</p> <p>---</p>	1-19
A	<p>JOURNAL OF POLYMER SCIENCE vol. 27, no. 11, 1989, pages 3745 - 3757</p> <p>E.J. VANDENBERG ET AL. 'THE SYNTHESIS AND SOLUTION PROPERTIES OF SOME RIGID-CHAIN, WATER-SOLUBLE POLYMERS...' see the whole document in particular pages 3746, 3755 and 3756</p> <p>---</p>	1-19
A	<p>WO,A,9 200 749 (MERRELL DOW PHARMACEUTICALS INC.) 23 January 1992 see abstract; claims; examples</p> <p>---</p>	1-19

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/06693

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

REMARK: Although claims 1-18 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2.  Claims Nos.:

1-19

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In view of the large number of compounds which are defined by the general formulas of claim 1 the search was limited to the inventive part of the molecule and to the compounds mentioned in the examples (Guidelines ..Part B, Chapt. II.7 last sentence and Chapt. III, 3.7)

3.  Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9306693  
SA 76927

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05/10/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0467185	22-01-92	AU-B-	635850	01-04-93
		AU-A-	8024291	09-01-92
		AU-A-	8286791	04-02-92
		CA-A-	2046491	10-01-92
		CN-A-	1058959	26-02-92
		EP-A-	0538373	28-04-93
		JP-A-	4226521	17-08-92
		WO-A-	9200749	23-01-92
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WO-A-9103226	21-03-91	AU-A-	6337590	08-04-91
		CA-A-	2064575	15-02-91
		EP-A-	0489102	10-06-92
		JP-T-	5503506	10-06-93
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GB-A-781479		None		
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WO-A-9200749	23-01-92	AU-B-	635850	01-04-93
		AU-A-	8024291	09-01-92
		AU-A-	8286791	04-02-92
		CA-A-	2046491	10-01-92
		CN-A-	1058959	26-02-92
		EP-A-	0467185	22-01-92
		EP-A-	0538373	28-04-93
		JP-A-	4226521	17-08-92
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